Toxicology Drug Screening in Blood by CE-MS^n - a Feasibility Study

Application

Forensics, Toxicology

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Abstract

Preliminary observations indicate the feasibility of capillary electrophoresis-mass spectrometry (CE-MS) with the Agilent LC/MSD Trap system to be capable of detecting drugs at <20 ng/mL in whole blood, suggesting that it may be a suitable tool for screening whole blood samples for drugs. Additionally, the capability of generating MS^n data permits reliable identification of detected drugs, suggesting that both screening and identification of drugs might be accomplished in one or two injections using the same instrument system.

Introduction

Capillary electrophoresis (CE) with ultraviolet-diode array detection (UV-DAD) is reported to be an effective tool in the screening of whole blood samples for drugs of toxicological interest [1]. The first objective of any such screen is simply the detection of drugs at toxicologically significant concentrations. Since the primary focus is on detection, drug identification is often tentative at the screening stage. Rigorous identification is frequently deferred to a second analytical procedure, such as mass spectrometry (MS), often involving repeat extraction and derivatization. Analysis is more efficient if both detection and identification can be completed as part of the same analytical run or, at least, using the same instrumentation.

It was reported that CE-MS is useful in the analysis of drugs of abuse in samples of illicit drug materials [2]. An earlier application note showed that CE-MS^n is capable of separating and detecting certain drugs with sufficient sensitivity to be useful as a possible screening instrument in whole blood analysis [3]. Initial observations were made using pure drug solutions. CE-MS can only be useful in the screening of whole blood, however, if residues from the matrix do not interfere. The present application note describes the analysis of certain drugs in samples of whole blood, both spiked samples and those from actual toxicology cases.

In the report of Hudson *et al* [1], it was noted that the combination of electrophoretic mobility and the UV spectrum provided an impressive degree of discrimination between analytes. This combination, however, was not claimed to represent rigorous identification. Rather, drugs tentatively identified by mobility and UV absorption would be subjected to further analysis, probably by gas chromatography/mass spectrometry (GC/MS). This note shows the feasibility of extending the CZE (Capillary Zone Electrophoresis) screening procedure put forward by Hudson et al [1] to include the collection of +MS, +MS^2 and +MS^3 data during a single screening run and the subsequent use of the MS^n data as evidence of drug identification.

Experimental

All drug analyses were done using an Agilent G1600A 3D CE coupled to an Agilent LC/MSD Trap XCT, using the G1603A CE-MS adapter kit and the G1607A CE-MS sprayer.

CE conditions

Capillary:	Bare fused silica; 50-µm diameter
Capillary length:	21.5 cm to DAD; 84.0 cm to LC/MSD Trap
Cassette temperature:	25 °C
Run buffer:	100 mmol/L phosphate at pH 2.38
Injection:	Electrokinetic, 12.0 kV for 16.0 s
Run voltage:	Ramped 0 to 20 kV in 0.15 s; held for duration of run
Run time:	25 min
Diode array:	Wavelength: 200 nm, bandwidth 4 nm
Reference wavelength:	375 nm, bandwidth 75 nm

LC/MSD Trap conditions

Mass range mode:	Ultra Scan
Ion polarity:	Positive
Ion source type:	ESI
Drying gas temp:	130 °C
Drying gas flow:	7.00 L/min
Nebulizer:	8–12 psi
Trap drive:	27.0
Skim 1:	40.0 V
Skim 2:	–5.0 V
Octopole RF amplitude:	131.2 V
Capillary exit:	97.0 V
Scan range:	Typically 50–500
Averages:	8 spectra
Max. accumulation time:	100000 µs
ICC target:	100000
Charge control:	On
Sheath liquid:	0.5 % Formic acid in 50/50 methanol/water
Sheath liquid flow:	7.5 µL/min (0.75 mL/min split 100:1)

Auto MS parameters

Auto MS^3:	On
Threshold auto MS^3:	2500
No. of precursors:	1
Fragmentation amplitude:	1.0 V
Isolation width:	4.0 *m/z*

Whole porcine blood spiked with 17 drugs, each at a concentration of 20 ng/mL of blood was previously extracted according to the procedure of Hudson [1]. Details are given in the cited reference but, briefly, basic drugs were extracted as follows: 1.0-mL whole blood + 0.2-mL concentrated ammonia + 5.0-mL 1-chlorobutane; shake; centrifuge; and evaporate to dryness. To the dry residue, add 30 µL of 10-mmol/L phosphate buffer; vortex; centrifuge briefly; transfer to sample cups and centrifuge again. Each residue was then screened by $CE\text{-}MS^n$, using the above analytical conditions. For each peak detected, MS^n data were collected.

Samples of whole human blood from three actual toxicology cases were previously extracted and analyzed by ELISA, GC-NPD, GC-ECD, and CE-DAD. Drugs detected in the blood samples were identified by GC/MS. The dry residue from each of these samples was reconstituted and analyzed by $CE\text{-}MS^n$, as above.

No attempt was made to optimize separation conditions in this work. The extraction residues left over from previous analyses were simply analyzed under conditions that approximated those reported by Hudson [1]. We observed (and report here) the coelution of certain drugs that Hudson *et al* were able to separate. As shown below, however, the ability to collect MS^n data makes these coelutions a less serious problem than might be supposed since the added selectivity of MS^n overcomes coelution problems observed with the less-selective DAD.

Results and Discussion

Figure 1 shows the total ion electropherogram (TIE) of the spiked porcine blood sample, along with the extracted ion electropherograms (EIEs) for each of the 17 drugs present. These data give preliminary indications on two important points: sensitivity of detection and interference from matrix.

Initially, it was thought possible that the analytical system used here would simply not be sensitive enough for a toxicological screen. From the data shown, however, it is clear that useful MS data can be collected at drug concentrations of 20 ng/mL in whole blood. It also appears that a thorough validation study would show that the limit of detection (LOD) for at least some drugs would be substantially lower than 20 ng/mL. This is probably adequate sensitivity for routine drug screening.

It was also considered possible that matrix components extracted from whole blood would overwhelm the analytical system and interfere irreversibly with collection of the MS data. Figure 1 suggests that matrix components offer no serious obstacles to MS analysis.

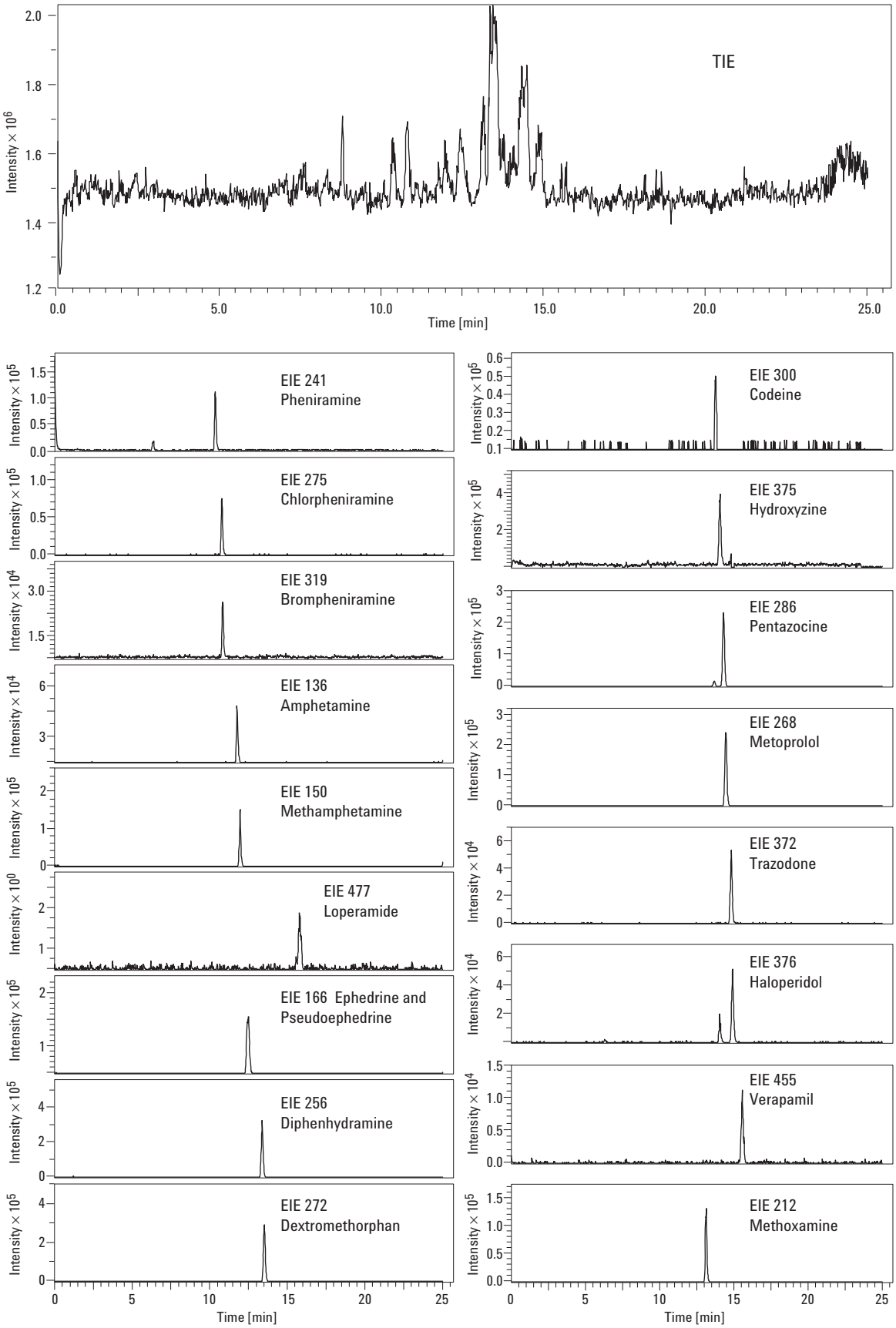

Figure 1. TIE and EIEs for extracted drug mix.

In Case 1, the blood sample was known from previous analyses to contain amphetamine, methamphetamine, and cocaine. Figure 2 shows the TIE and EIEs; Figure 3 shows Auto MS^n and library matches from the same sample.

Figure 2 shows one matrix peak (unidentified). As was suggested above, matrix interference does not appear to be a significant problem.

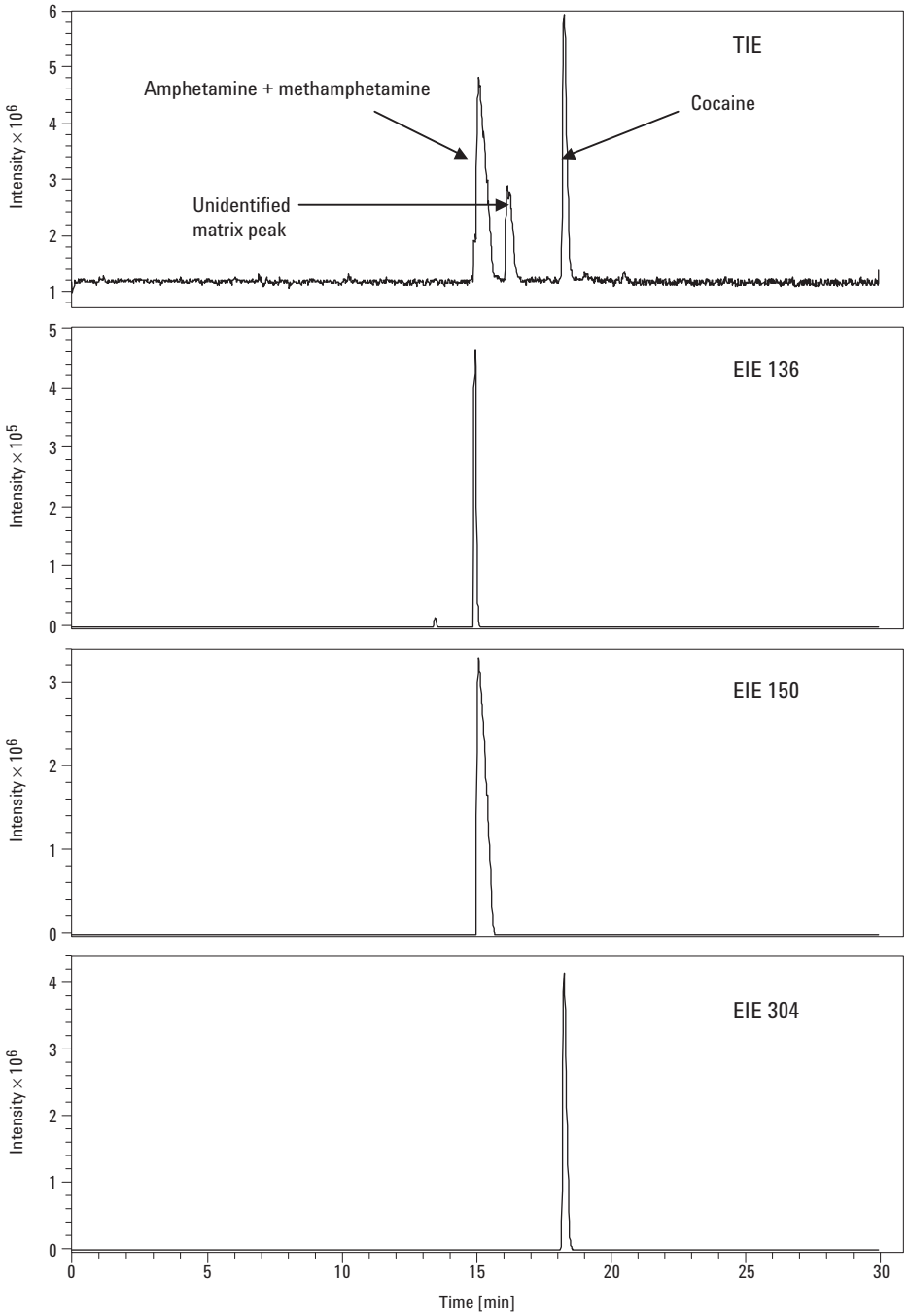

Figure 2. Case 1. Amphetamine, methamphetamine, and cocaine.

The library searches shown in Figure 3 indicate the correct identification of amphetamine, methamphetamine, and cocaine, in spite of the coelution of the former two compounds. The library search on MS^3 data from Peaks 1 and 2 (amphetamine and methamphetamine, respectively) showed no matching spectrum. The dominant ion in the library MS^3 data from both these compounds was 91.3 *m/z*. Our analysis showed a dominant ion at *m/z* 92.2, which was, of course, not interpreted as a match by the search software.

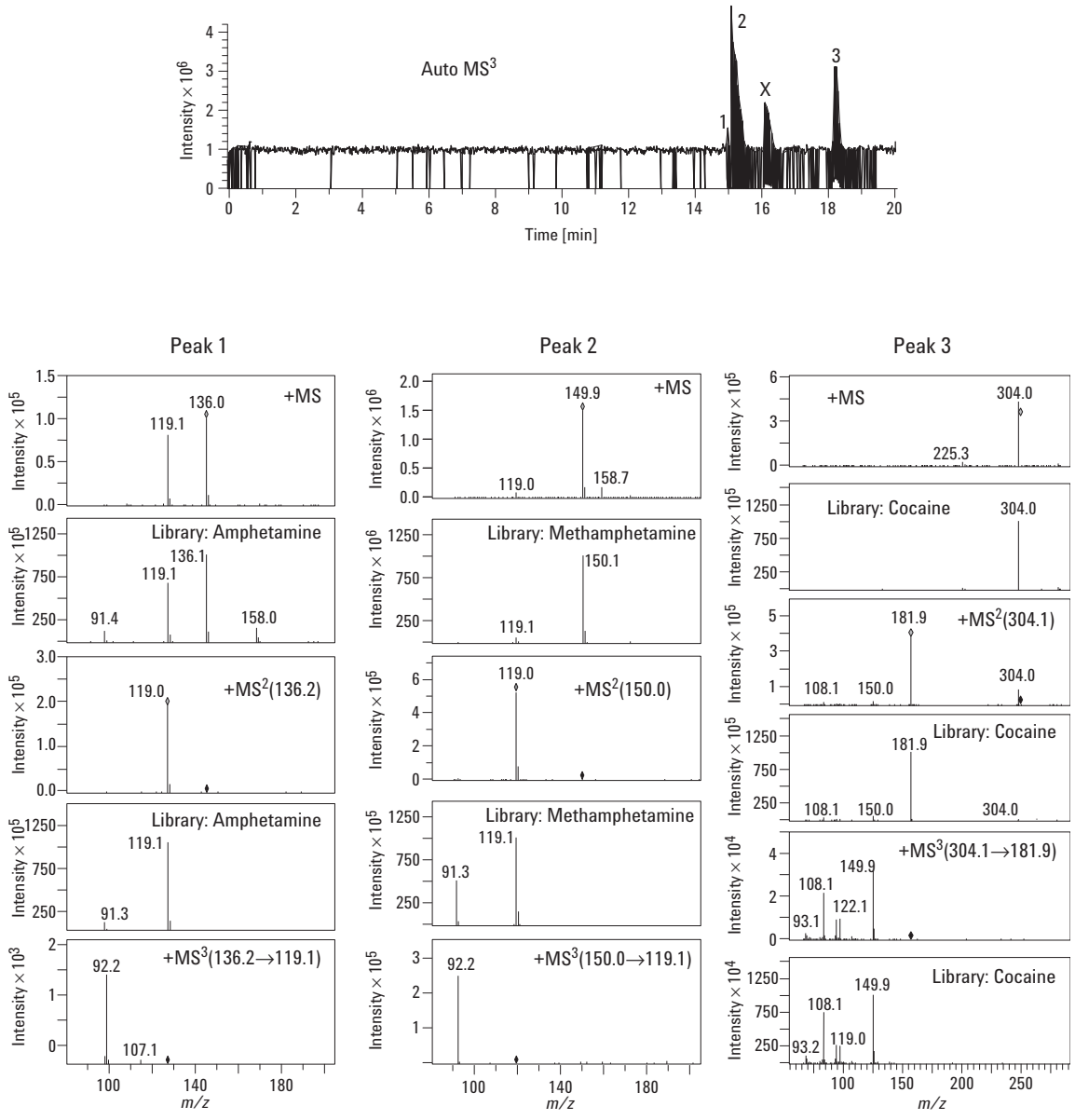

Figure 3. Case 1. AutoMS³ and library matches.

In Case 2, the blood sample was known to contain methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). Figure 4 shows the TIE and EIEs. Figure 5 shows the Auto MS^3 and library matches from the same sample.

Figure 4 shows again the relative absence of matrix interference and the coelution of the two analytes of interest.

The library searches shown in Figure 5 identified both drugs correctly. It is interesting to note, however, that the search of MS data on MDA failed to find a match but that the correct match was found for MS^2 and MS^3 data. This was attributed to the presence of background data, such as the fragments at *m/z* 91.1 and 158.8. In the analysis of MDMA, these background fragments were removed and correct matches were found on all three searches.

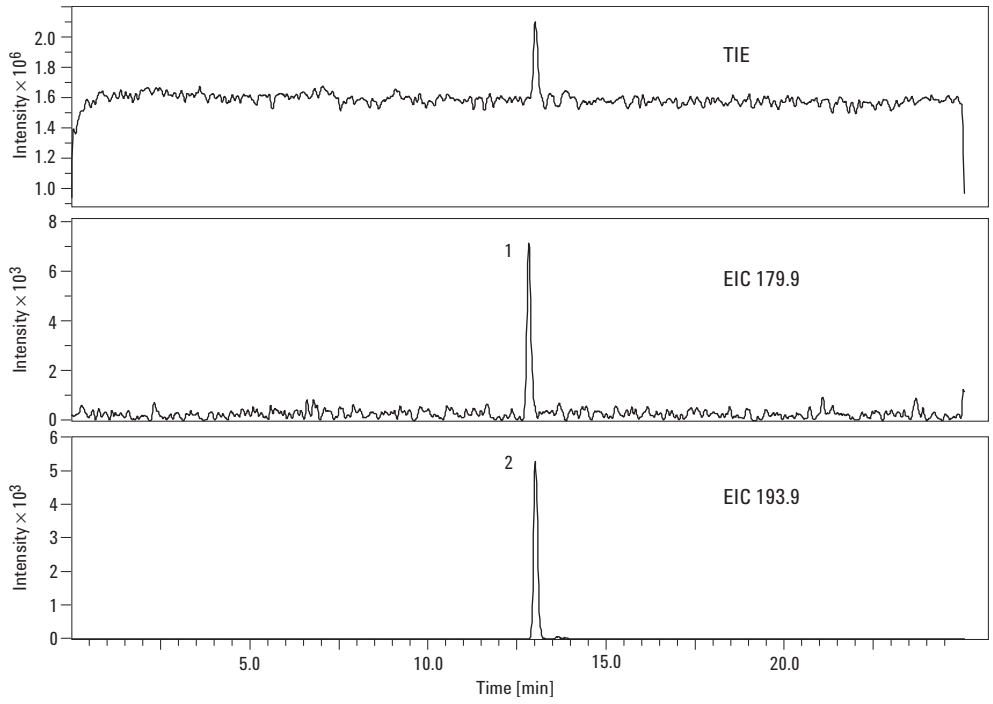

Figure 4. Case 2: MDA and MDMA.

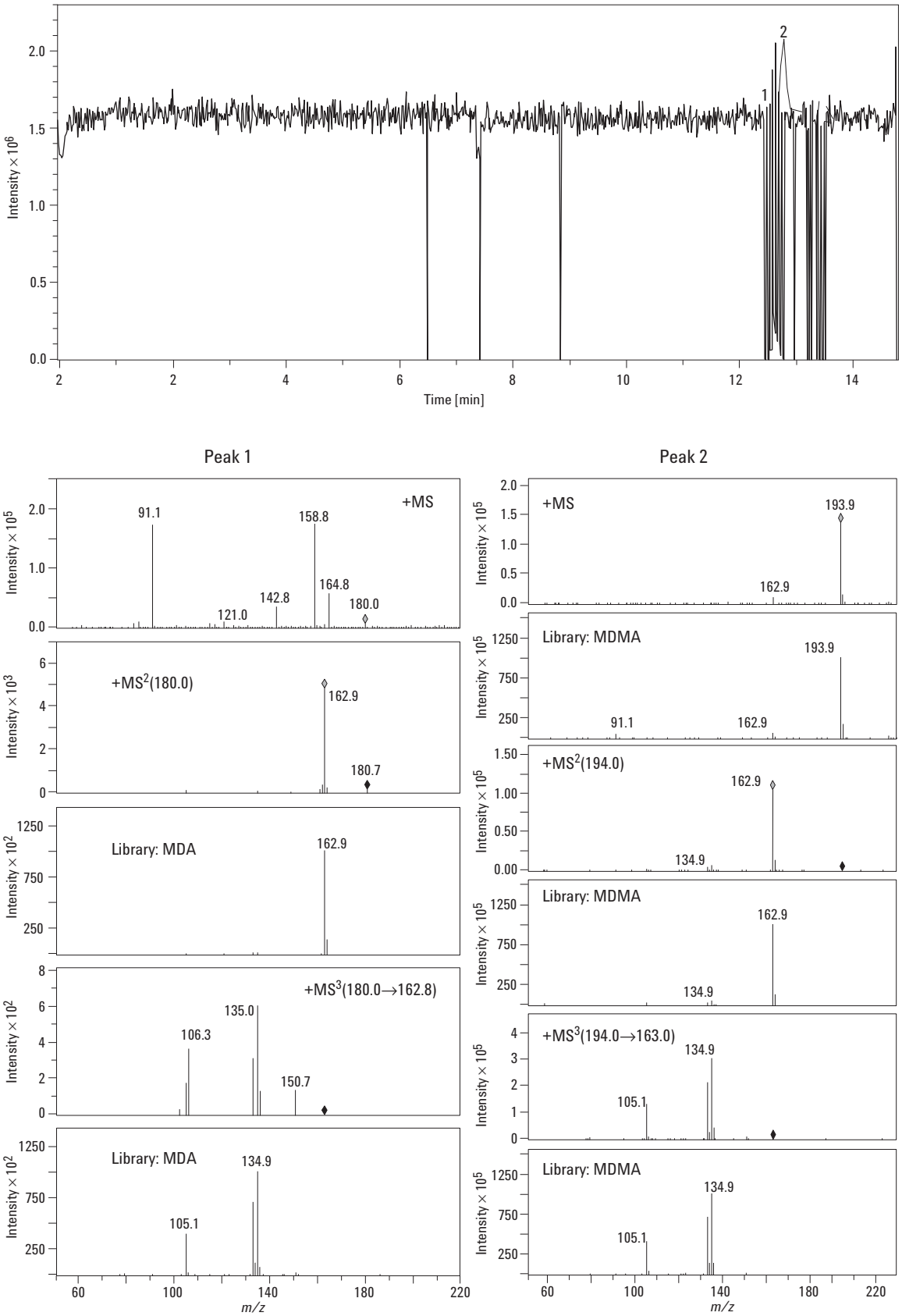

Figure 5 Case 2. Auto MS³ and library matches.

In Case 3, initial analysis of the blood sample by Hudson *et al* showed several forensically significant compounds. Quinine, cocaine, cocaethylene, bupropion, paroxetine, and lidocaine were identified by GC/MS. As before, our analysis of this sample attempted to replicate this work, given different instrument configurations. Figure 6 shows the TIE for this sample. The library matches from the Auto MS^3 analysis are shown in Table 1. There are several points to note in the following data.

First, for a variety of reasons, Peaks 2, 3, 4, and 5 were not included in Table 1. Peak 2 appeared upon later searching to be methylecgonine (see below) and Peak 3 appeared to be a matrix peak (unidentified); Peak 4 was consistent with the ISTD (methoxamine) and Peak 5 was consistent with the lidocaine metabolite, monoethylglycinexylidide (MEGX).

Second, erythrohydrobupropion and threohydrobupropion are known to be major metabolites of bupropion [4]. While Figure 6 suggests that these two compounds are potentially separable (Peaks 8 and 9), no attempt was made here to further resolve the system. As it was, the compounds were not separated by Auto MS^3 and, accordingly, Table 1 shows an entry only for Peak 8 thus indicating that the two compounds were indistinguishable on the basis of MS data. None of the metabolites of bupropion were previously identified by GC/MS.

Finally, with Peak 6, the known drug was not identified on the library search of MS data because of the presence of background fragments. As with the MDA/MDMA example in Case 2, however, identification was correct on the searches of MS^2 and MS^3 data.

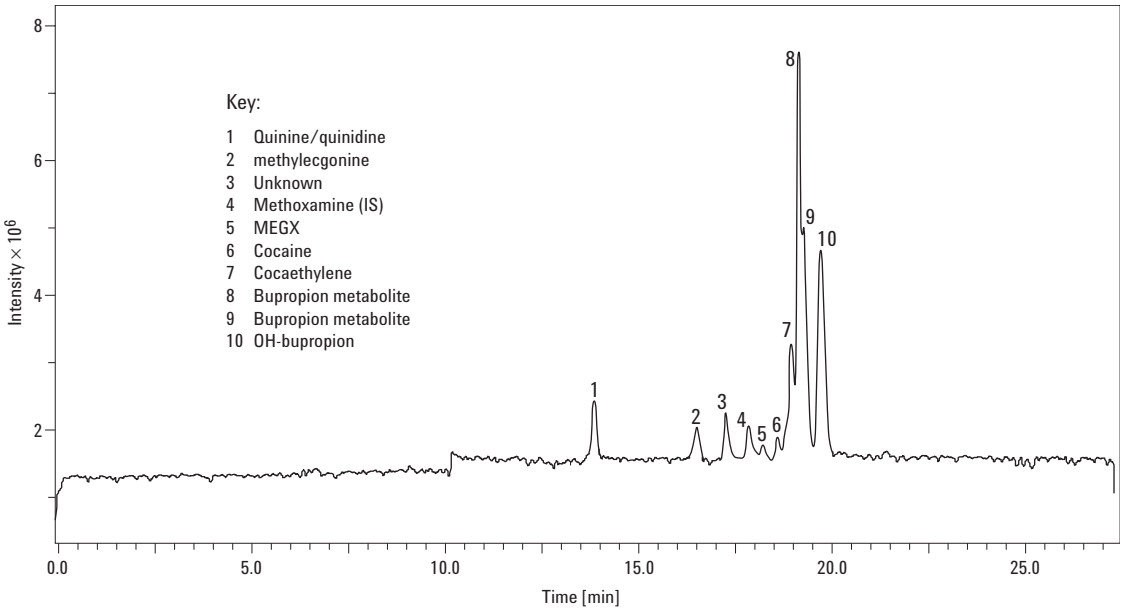

Figure 6. TIE of Case 3.

Table 1. Library Searches of Auto MS from Case 3

Identification	+MS	+MS^2	+MS^3	Fit	Rfit	Purity	Identification
Peak 1	325.2	307.1					
Library search	325.1			940	669	645	Quinine/Quinidine
		307.2		975	975	972	Quinine/Quinidine
			NC				
Peak 6	226.8 (Bkgd)	304.1	181.9	149.9			
Library search	304.1						No match
		181.9		995	995	995	Cocaine
			149.9	930	933	930	Cocaine
Peak 7	318.1	195.9	149.9				
Library search	318.1			994	992	992	Cocaethylene
		195.9		997	997	997	Cocaethylene
			149.9	997	998	997	Cocaethylene
Peak 8	241.9	167.9	150.9				
Library search	242.0			995	995	994	Amino OH bupropion*
		167.9		997	997	997	Amino OH bupropion*
			150.9	993	993	993	Amino OH bupropion*
Peak 10	256.0	237.9	138.9				
Library search	256.0			994	998	992	Hydroxybupropion
		237.9		998	998	998	Hydroxybupropion
			138.9	994	994	994	Hydroxybupropion

NC Not completed.

*Metabolites of bupropion exist in erythro- and threo- forms, not completely resolved here.

The AutoMS^3 analysis shown above did not detect certain compounds, such as bupropion, that were known to be present. Whether a particular peak is detected by AutoMS^3 or not depends upon several factors, chief among them the AutoMS^n threshold that is set and the resolution that is possible with the electrophoretic conditions used. Note that it is possible to prepare an Include List specifying precursor ions that the analyst specifically wants to search for. That was not done in this work.

However, later manual searching of the electropherogram (Figure 6) on the basis of selected masses indicated the presence of lidocaine and its metabolite, MEGX, in the region of Peak 5. Bupropion, metabolites of which were detected by AutoMS^3 in Peaks 8 and 10, appeared to be part of the unresolved complex at Peak 8. While both cocaine and the metabolite, cocaethylene, were detected by AutoMS^3, methylecgonine was not. A search based on its mass showed methylecgonine likely to be present in Peak 2. It was not detected in the AutoMS^3 analysis because we had no entry for it in our library.

Conclusions

CE-MS with the Agilent LC/MSD Trap system is capable of detecting drugs at concentrations less than 20 ng/mL in whole blood. It is, therefore, a suitable tool for screening whole blood samples for drugs as part of routine toxicological analyses. In addition to relatively sensitive detection, however, the capability of generating MS^n data permits reliable identification of detected drugs. This means that both screening and identification of drugs might be accomplished in one or two injections in a single instrument system. Finally, we show that, through the use of the Auto MS^n feature, it may be feasible to automate much of the drug screening procedure, thereby potentially increasing sample throughput significantly. We emphasize that these are preliminary observations indicating feasibility only. More development and validation work is required.

References

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